What do we know about pollutants that can affect ecosystems and human health?

Increasing concern that agriculture runoff and by-products from industry may be having adverse effects on the environment

Example:

REPRODUCTIVE HEALTH OF BASS IN THE POTOMAC, USA, DRAINAGE: PART 2. SEASONAL OCCURRENCE OF PERSISTENT AND EMERGING ORGANIC CONTAMINANTS

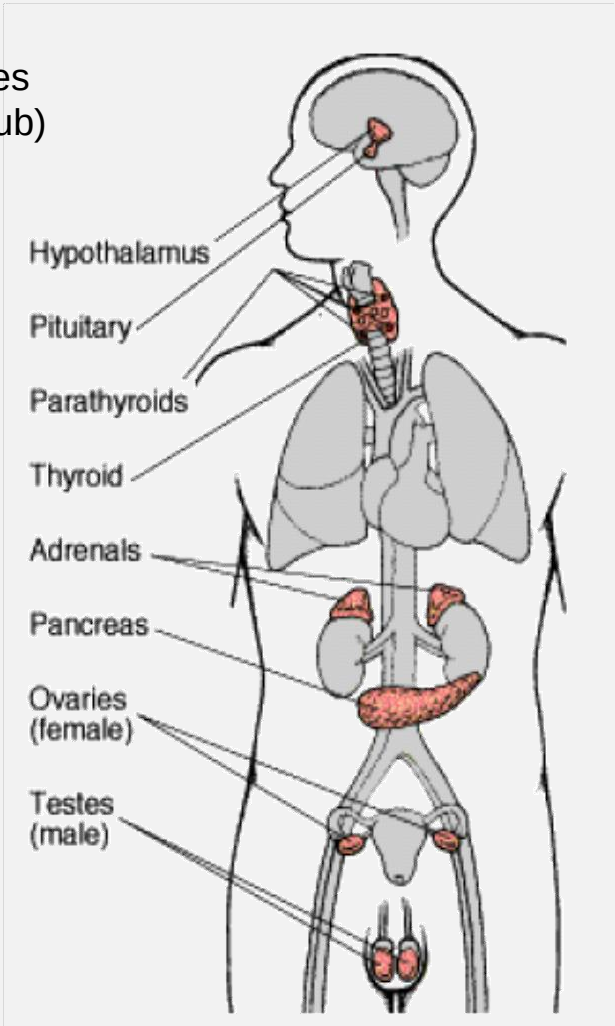
DAVID A. ALVAREZ,*† WALTER L. CRANOR,† STEPHANIE D. PERKINS,† VICKIE L. SCHROEDER,‡ LUKE R. IWANOWICZ,§ RANDAL C. CLARK,† CHRISTOPHER P. GUY,‖ ALFRED E. PINKNEY,‖ VICKI S. BLAZER,§ and JOHN E. MULLICAN#

†U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, Missouri 65201
‡Arctic Slope Regional Corporation Management Services, 4200 New Haven Road, Columbia, Missouri 65201, USA
§U.S. Geological Survey, National Fish Health Research Laboratory, 11649 Leetown Road, Kearneysville, West Virginia 25430
‖U.S. Fish and Wildlife Service, Chesapeake Bay Field Office, 177 Admiral Cochrane Drive, Annapolis, Maryland 21401
#Maryland Department of Natural Resources, Fisheries Service, Lewistown Work Center, 10932 Putman Road, Thurmont, Maryland 21788, USA

Endocrine Disruptors

Background:

Endocrine Disruptor (ED) exogenous substances or mixtures that alter function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations (WHO/I 2002)



Characteristics of EDCs

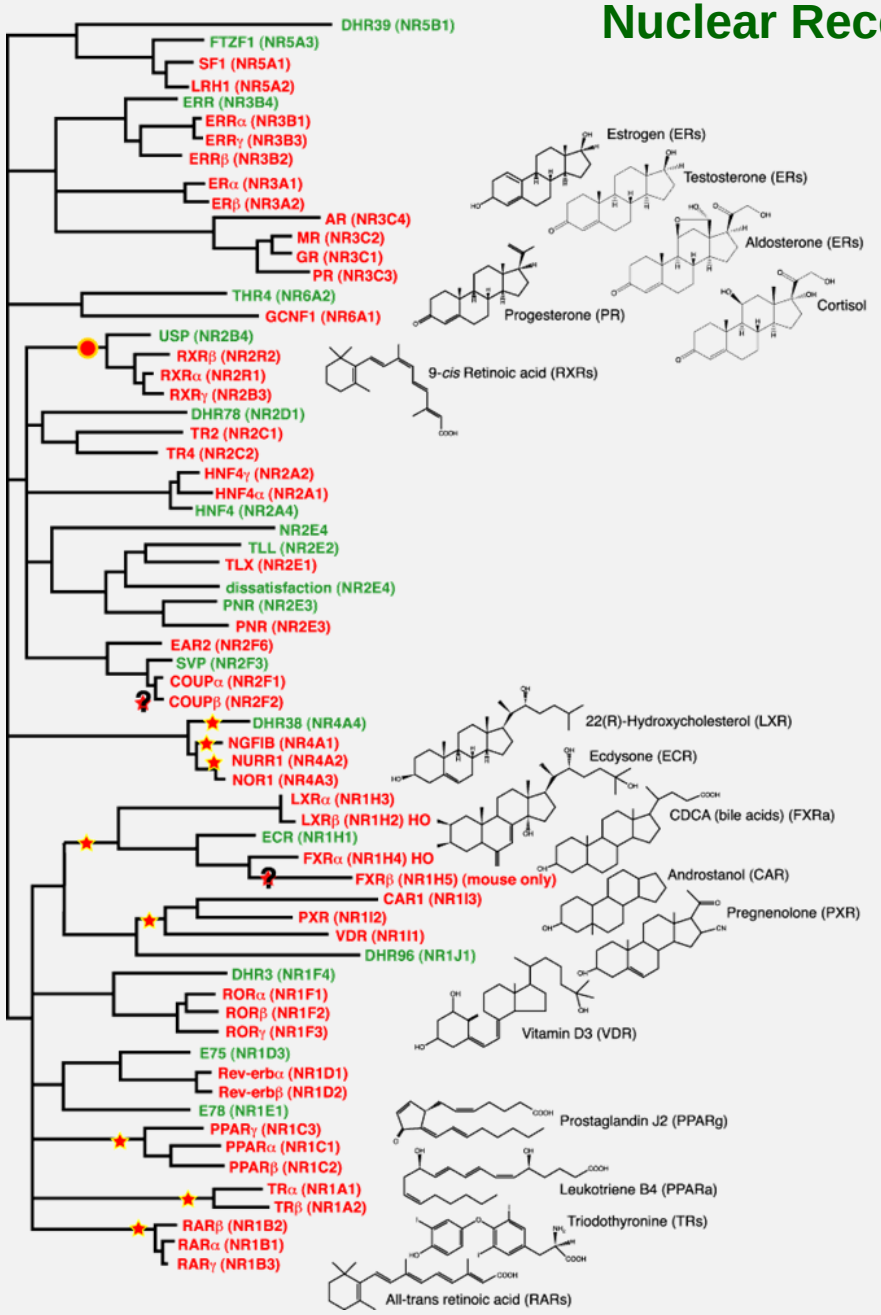
• Low dose effects
– High dose effects are different from low dose effects
– Non-monotonic dose responses

• Wide range of effects
– Endocrine signaling governs all tissues/organs
– Nuclear and membrane receptors, neurotransmitters, etc.

• Persistent and latent effects
– Developmental exposure most sensitive window
– Transgenerational effects (dioxin, BPA, phthalates)

• Ubiquitous exposure
– Consumer products
– Pharmaceuticals
– Industrial products

Nuclear Receptor Superfamily



These receptors are:

Major players in human endocrine regulation

and,

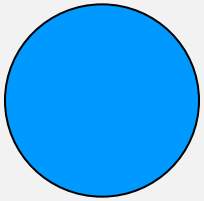
obvious targets for EDCs

Drosophila

21 receptors

Homo sapiens

48 receptors



C. elegans

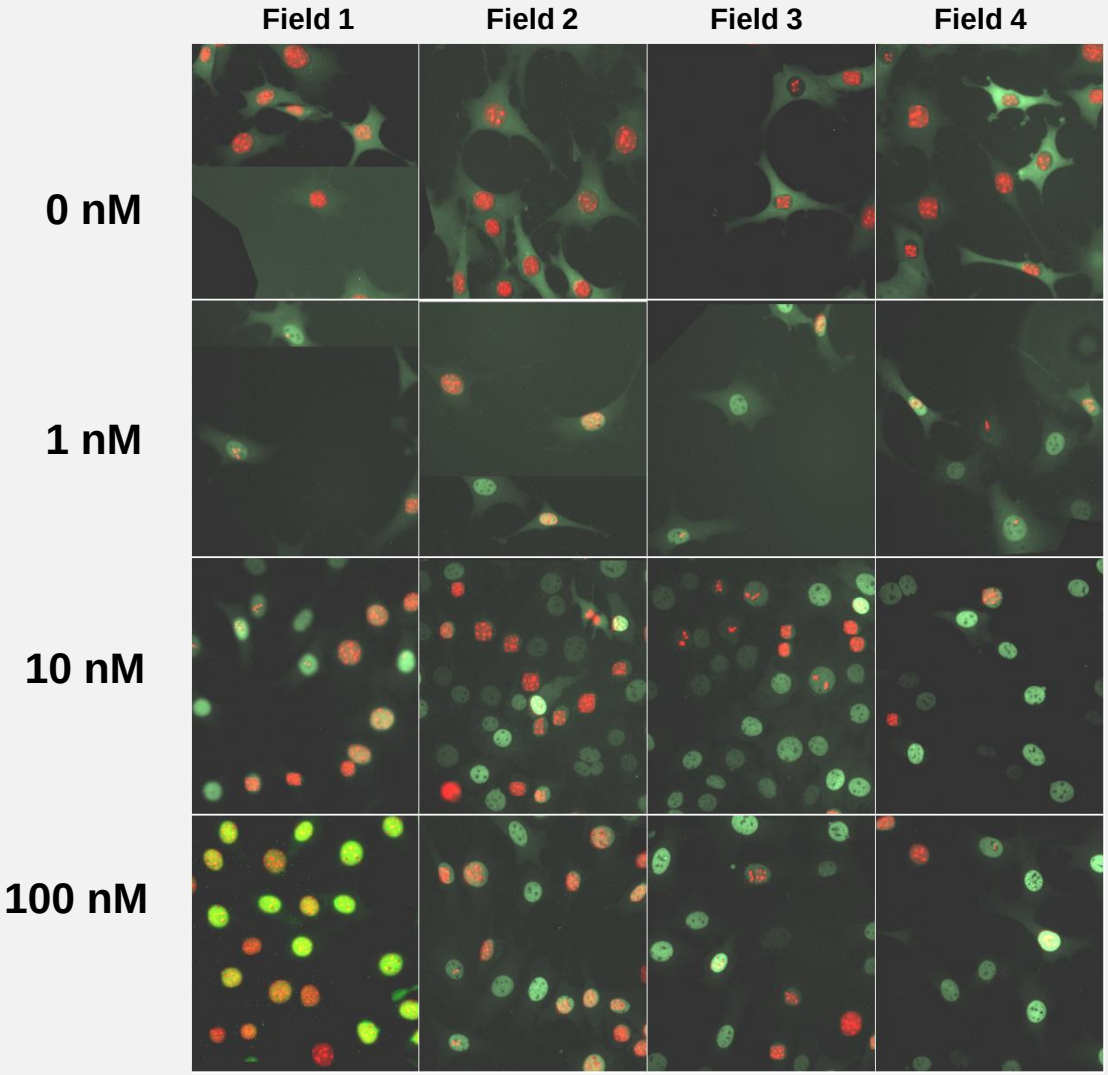
270 receptors

J Cell Sci 116:585 (2003) Laudet & Colleagues

How can we detect potential NR ligands in the environment?

Most nuclear receptors are constitutively present in the nucleus

Some nuclear receptors display ligand-dependent subcellular redistribution



When the receptor is fluorescently tagged, this property can be used to detect hormone concentrations in samples of interest

Red = Dapi

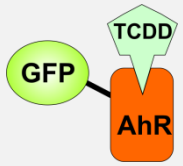
Green = GFP-GR

Example for the Glucocorticoid Receptor

Representative Fields

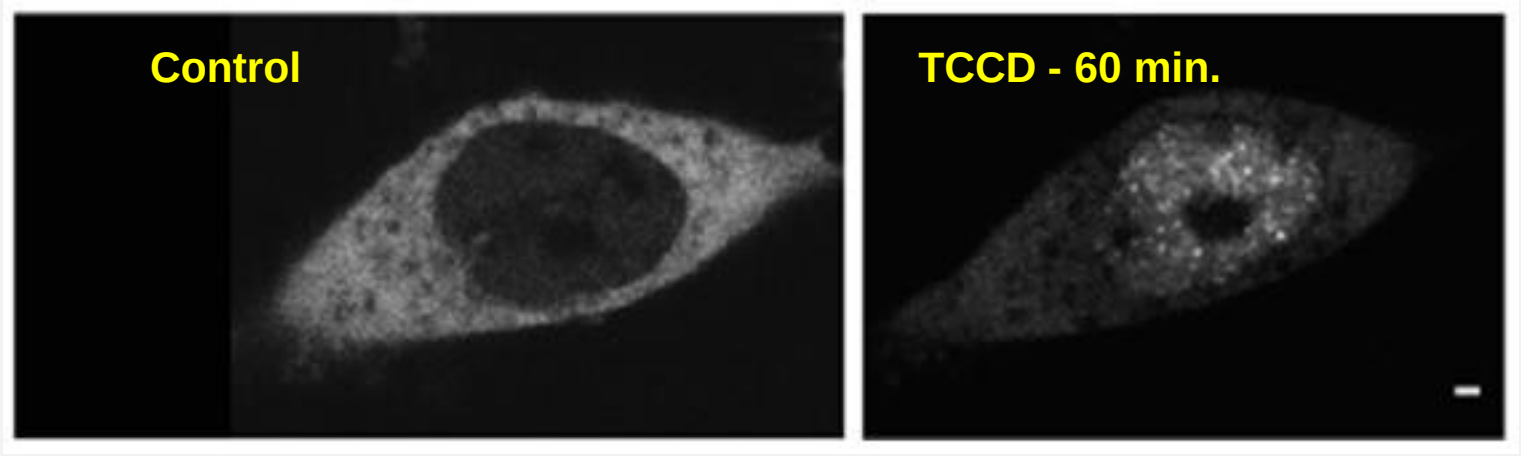
Other examples

GFP-AhR



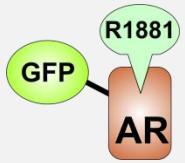
“dioxin receptor”

aryl hydrocarbon receptor

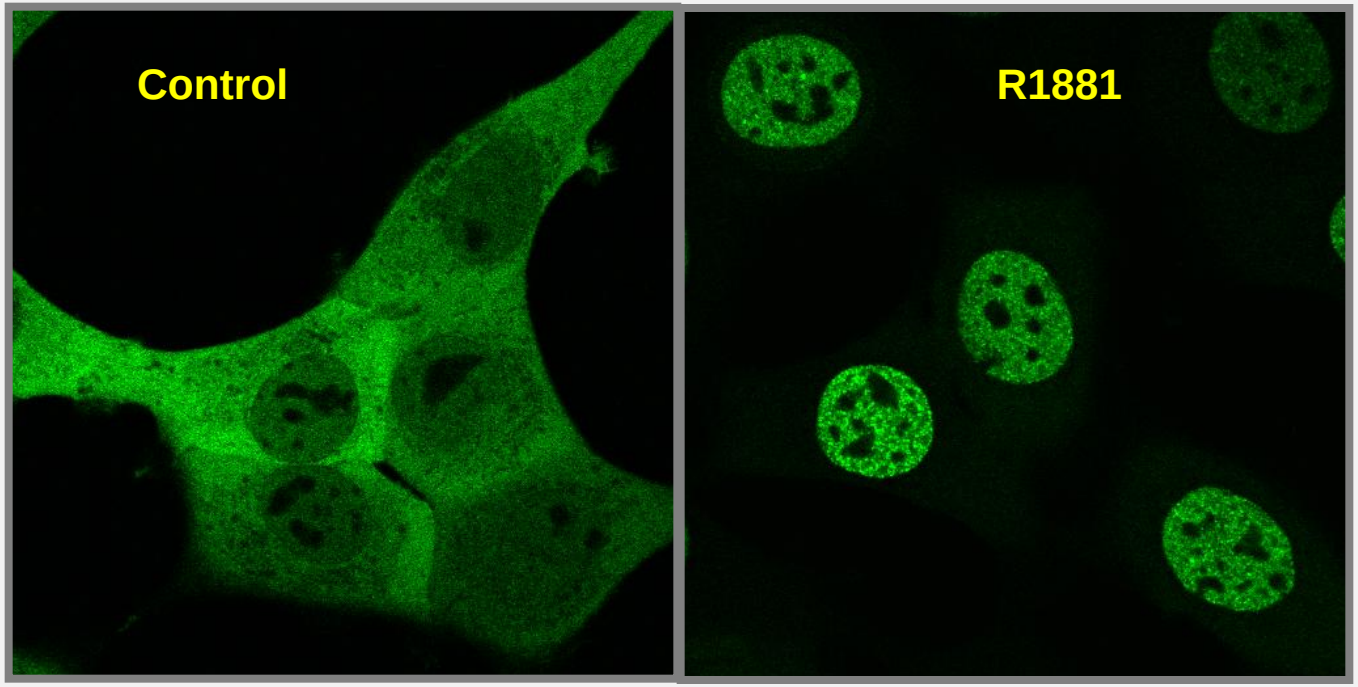


Other examples

GFP-AR

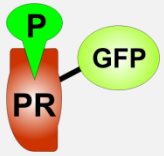


androgen receptor

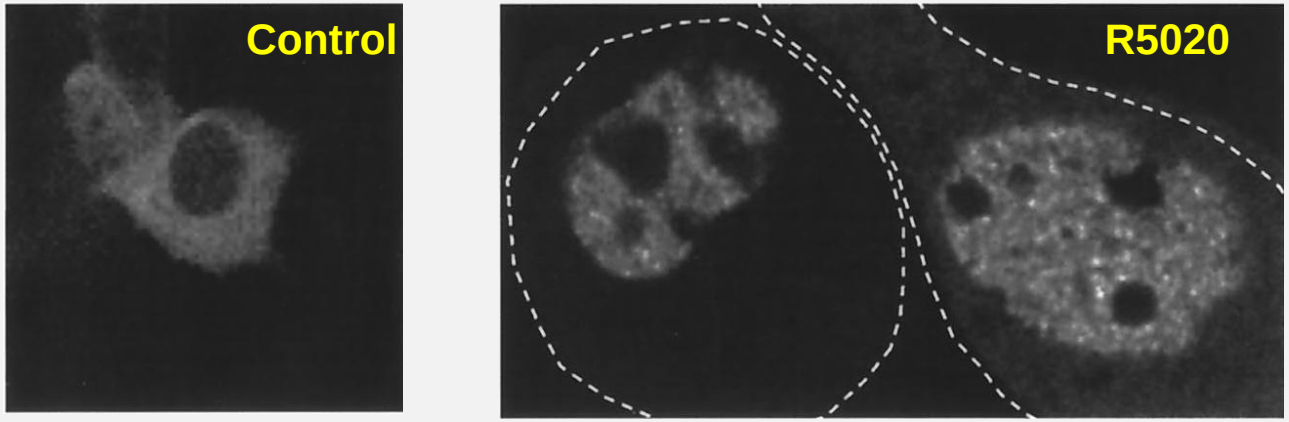


Other examples

GFP-PR



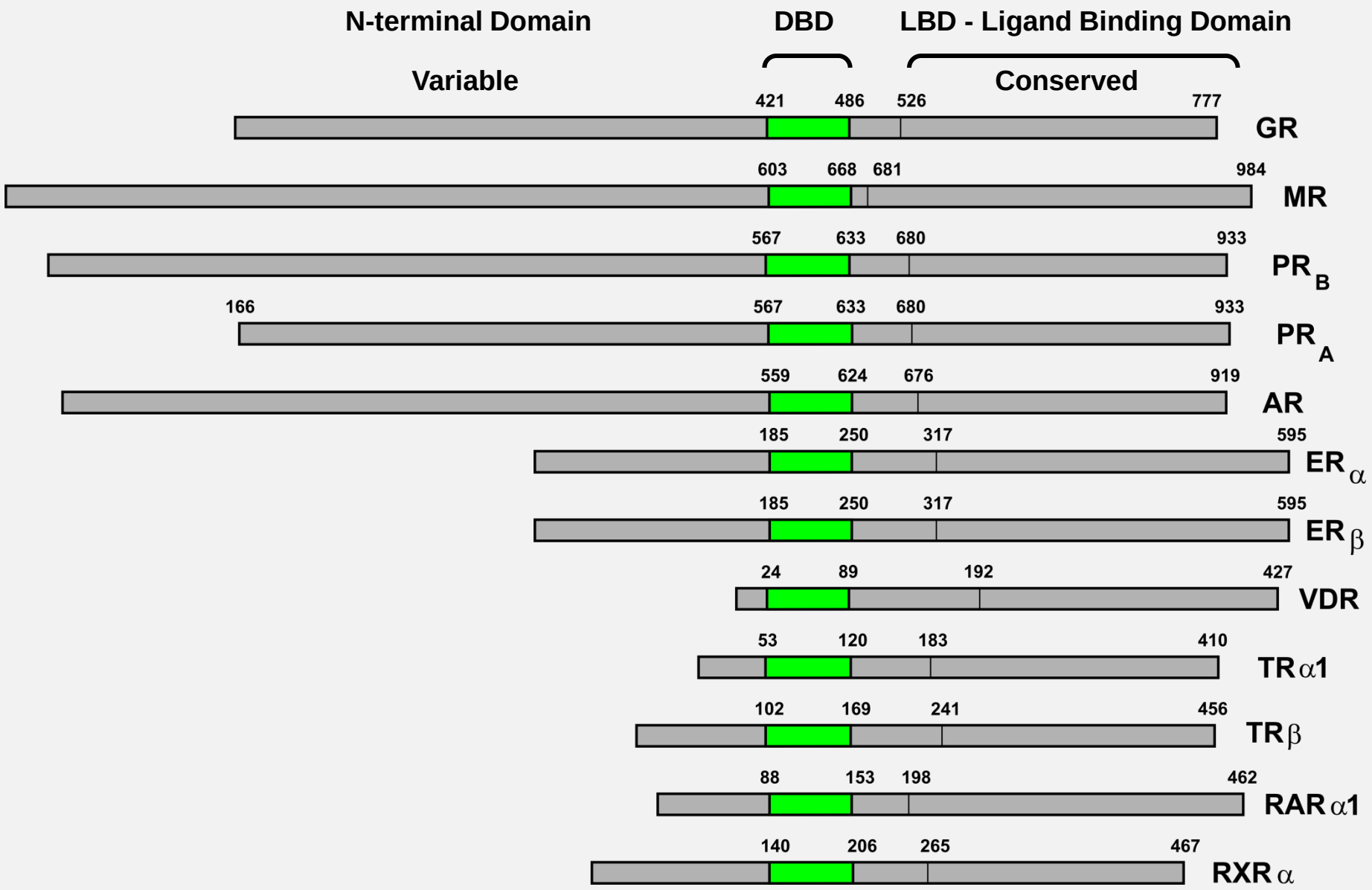
Progesterone receptor B



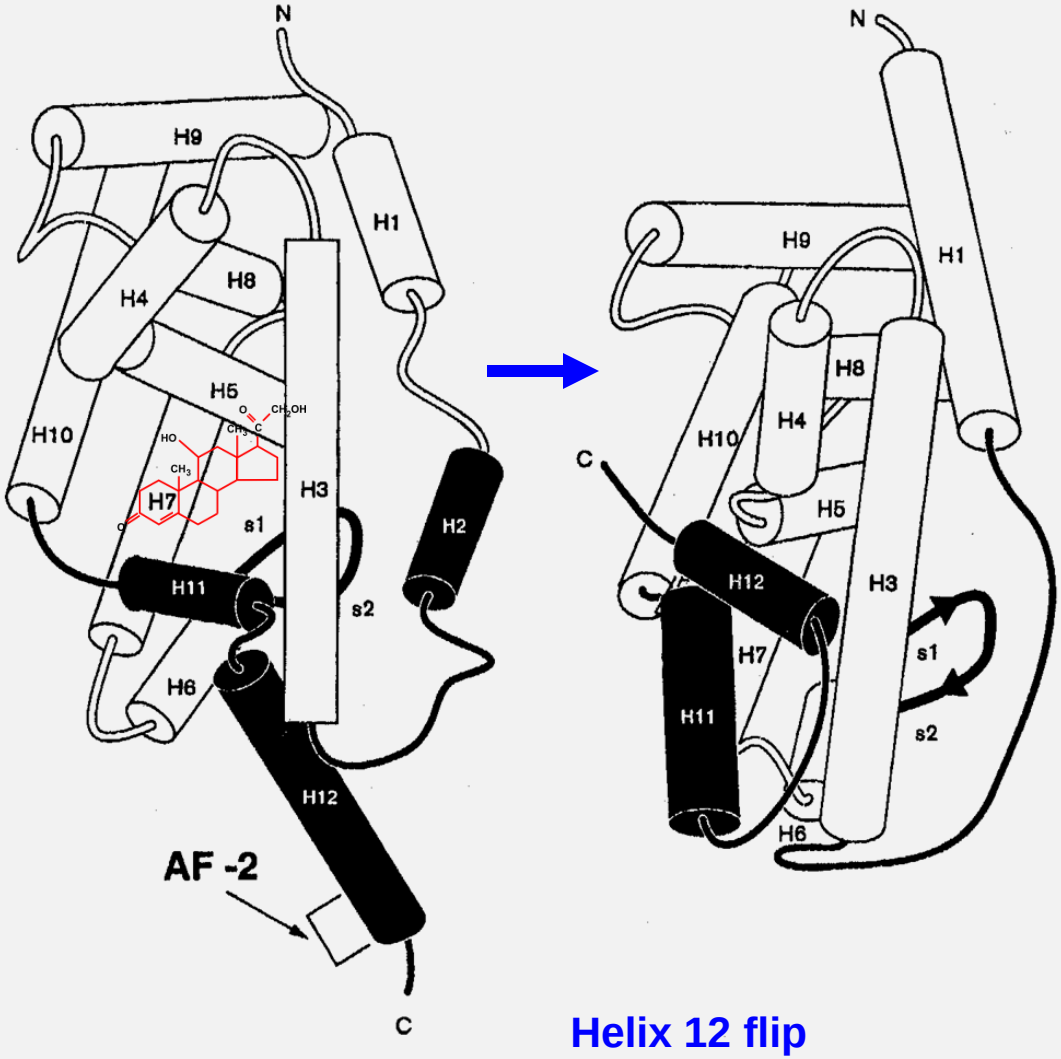
Progesterone receptor A form is in the nucleus minus ligand

What about receptors that do not translocate in response to ligand?

Steroid/Nuclear receptors have a highly conserved domain structure



Nuclear Receptor Ligand Binding Domain is a conserved 12 helix bundle

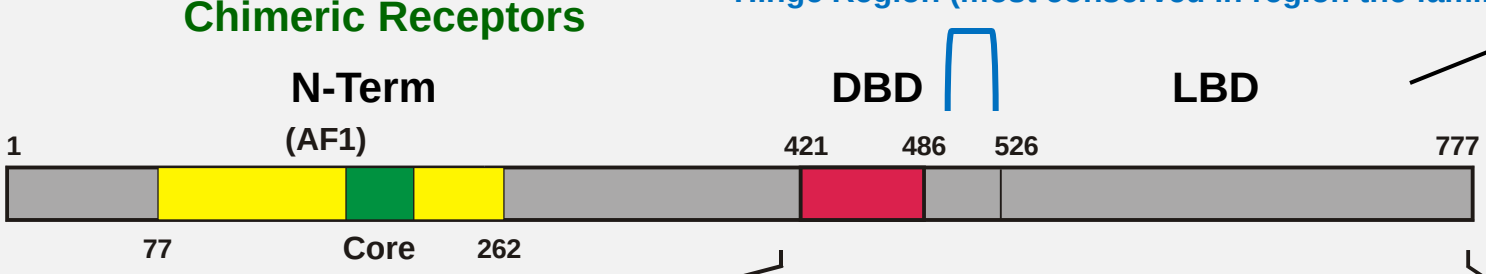


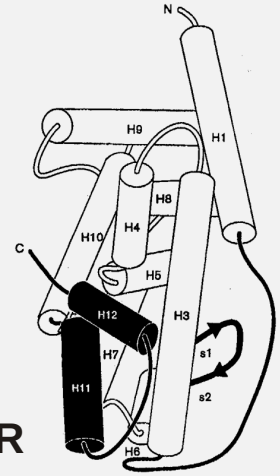
Helix 12 flip

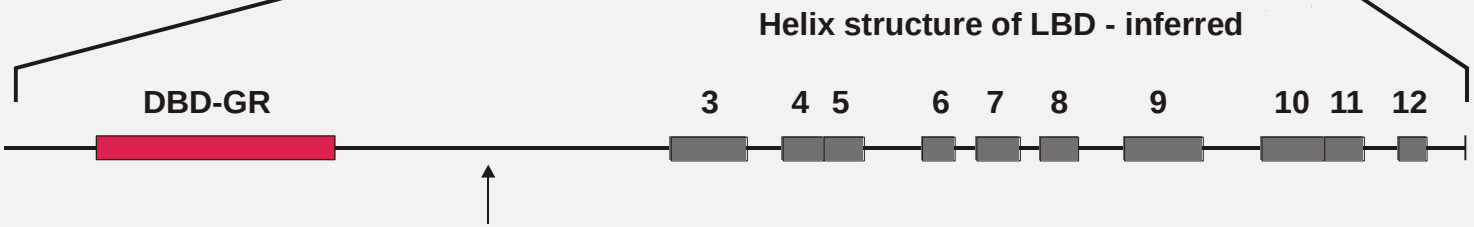
LBD conservation allows engineering of family members

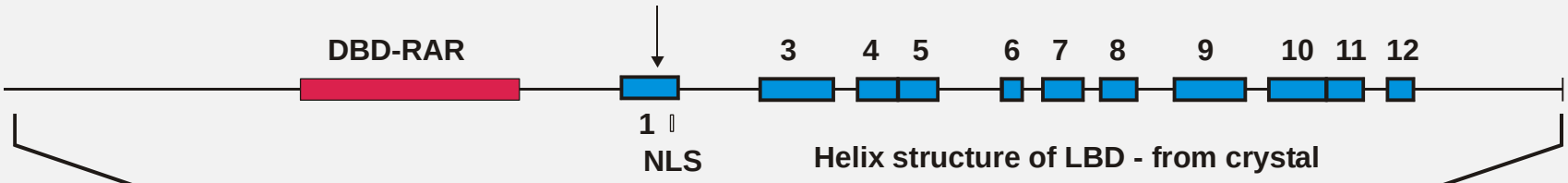
Take the LBD from a non-translocating receptor
Fuse to a translocating version at the hinge region

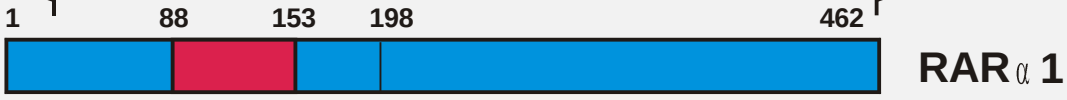
Chimeric Receptors





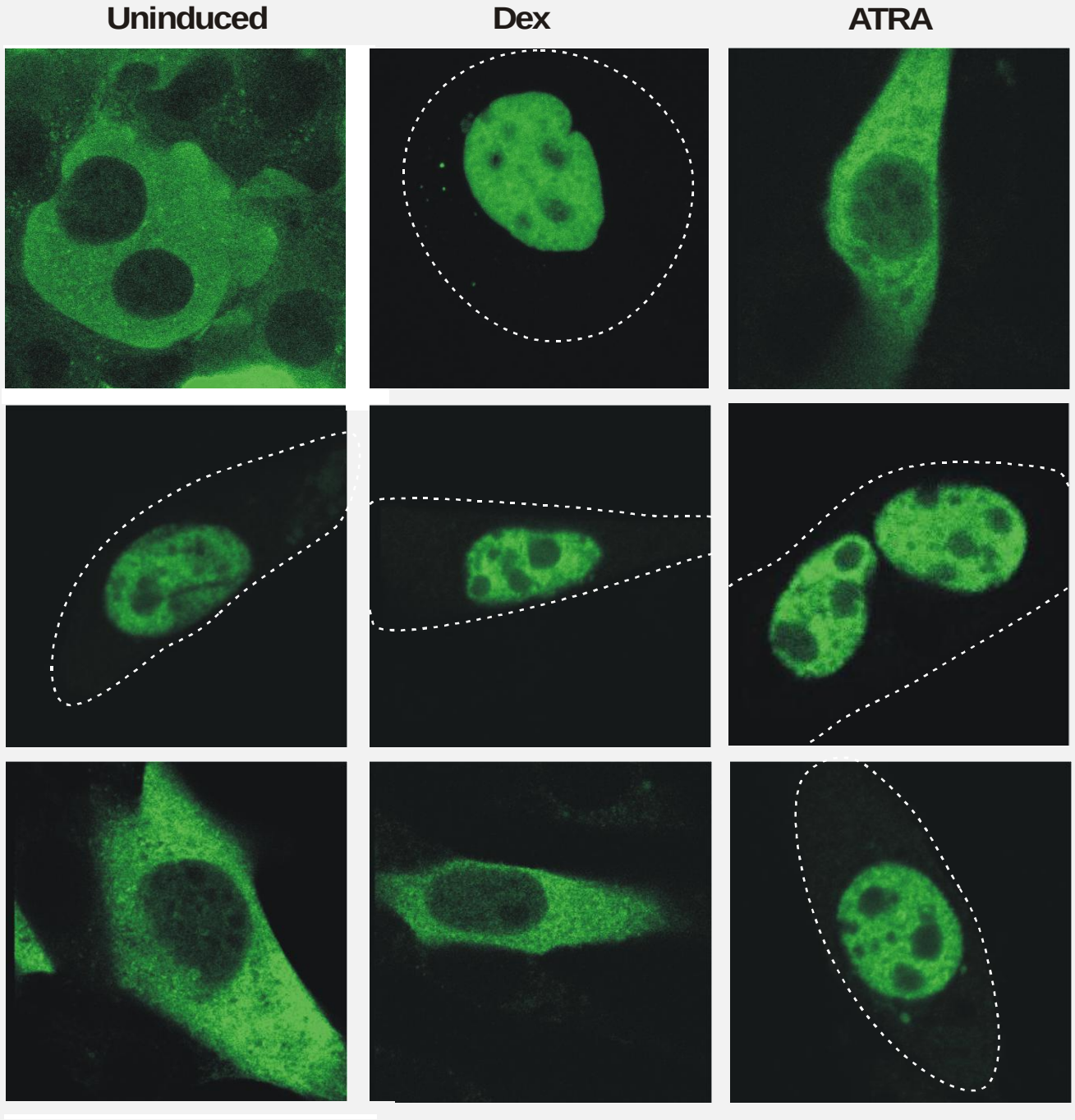






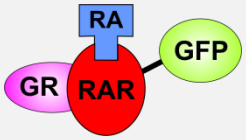
Fusion chimera will adopt the translocating property

Chimeric GR-RAR

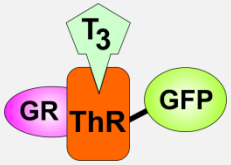


These chimeric fusions open the possibility of a general assay principle for receptor ligands in the environment

GFP-GR-RAR

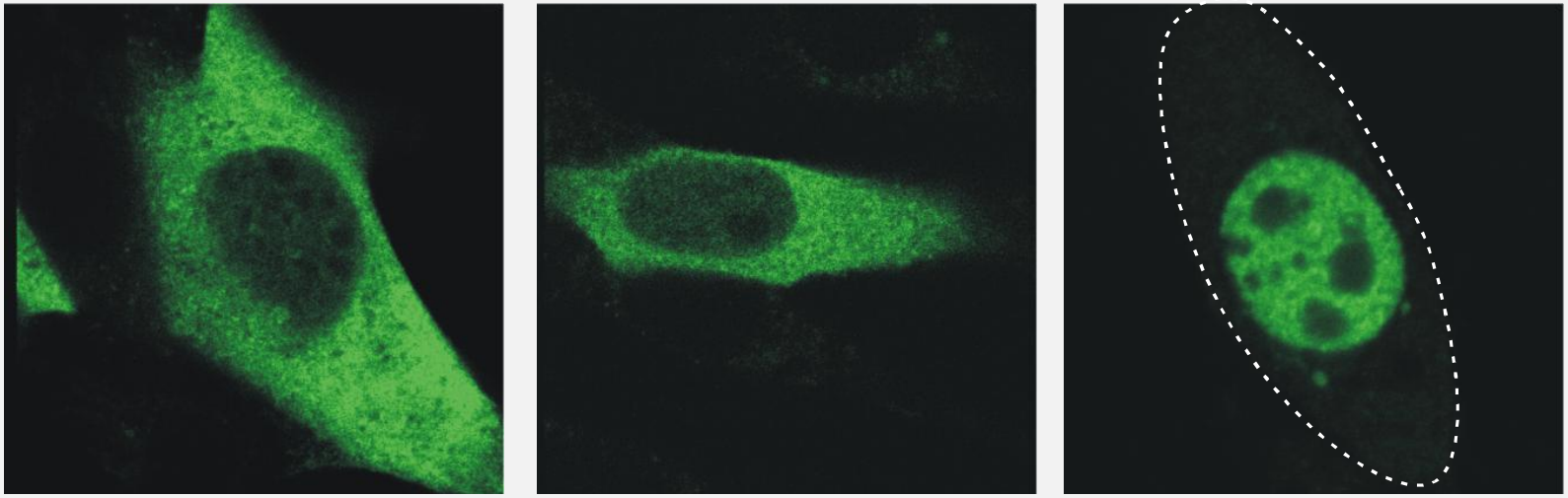


(recently established for ThR)



Retinoic acid receptor (GR – chimera)

GR-RAR



A High-Throughput Assay for Detection and Monitoring of Endocrine-Disrupting Chemicals in Water Sources

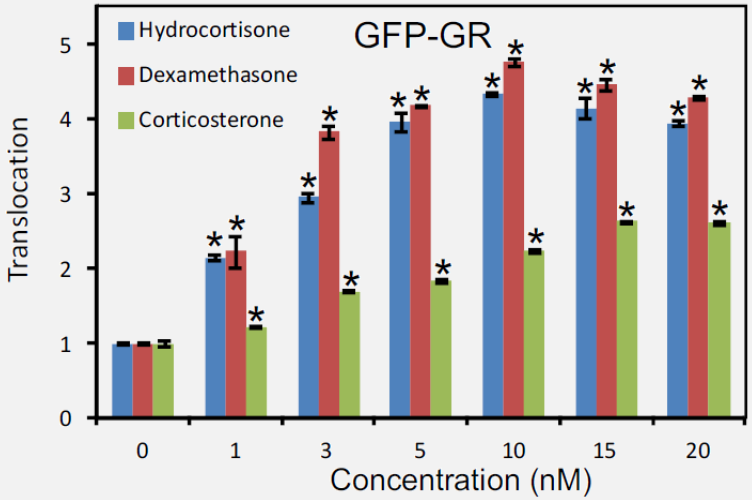
Diana Stavreva

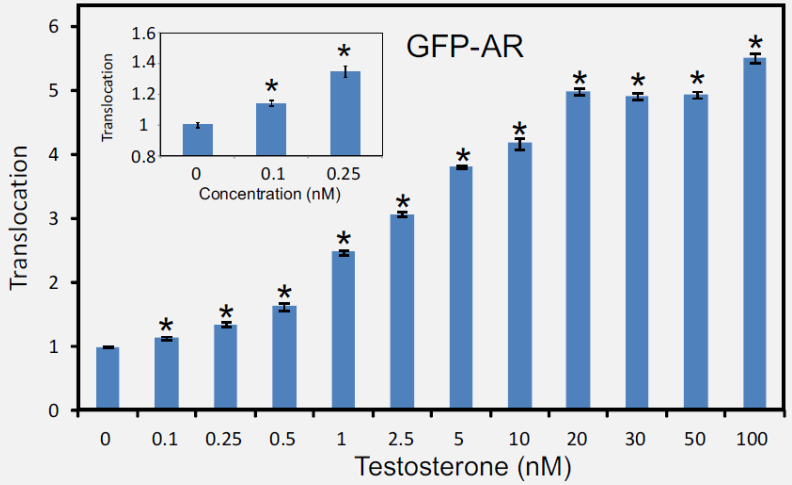
Lyuba Varticovski

Collaboration with Luke Iwanowicz, USGS-BRD

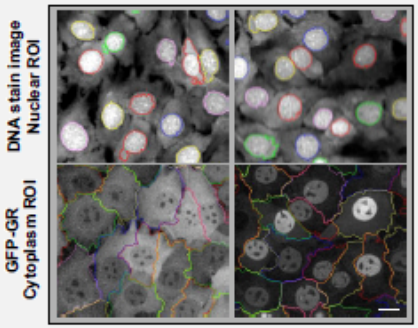
Automated imaging analysis system (Opera)

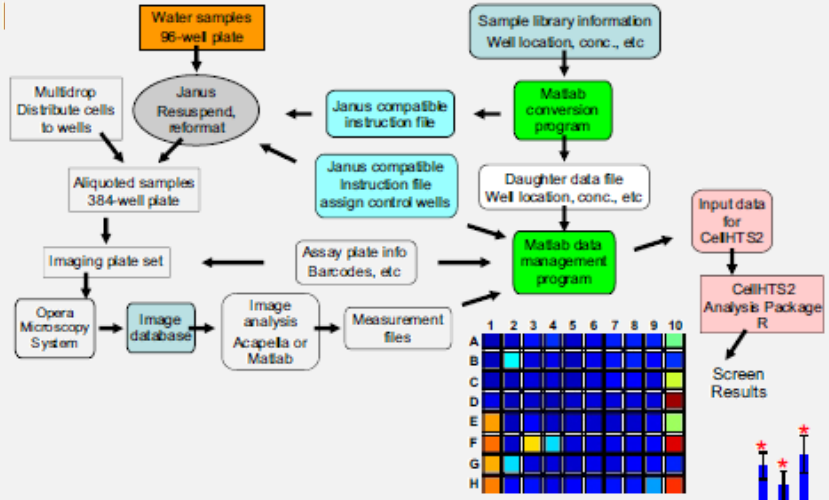
Concentration-dependent translocation of GFP-GR and GFP-AR in response to known hormones

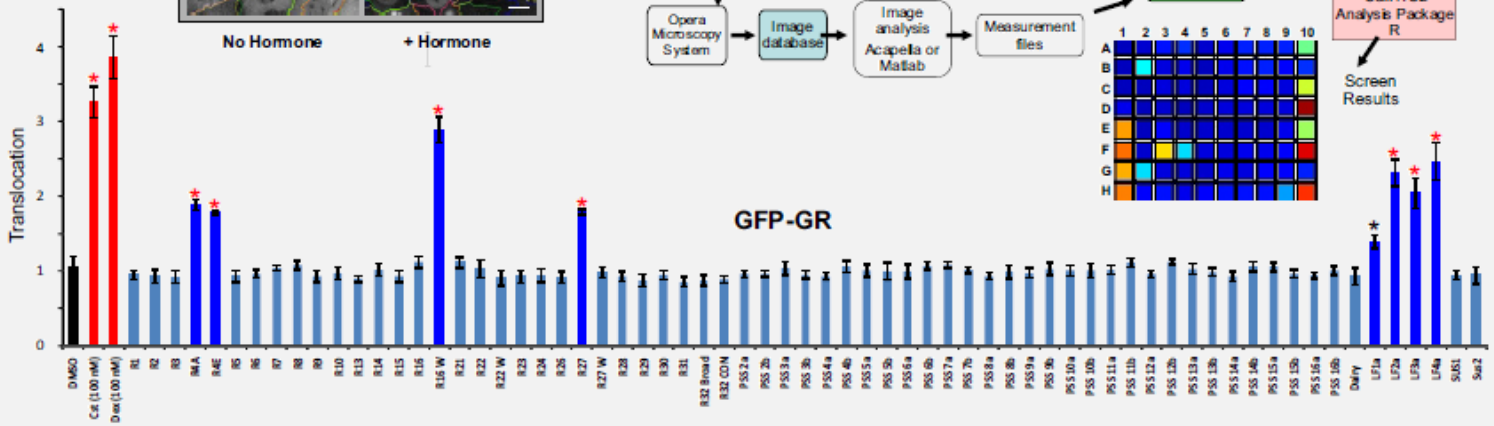


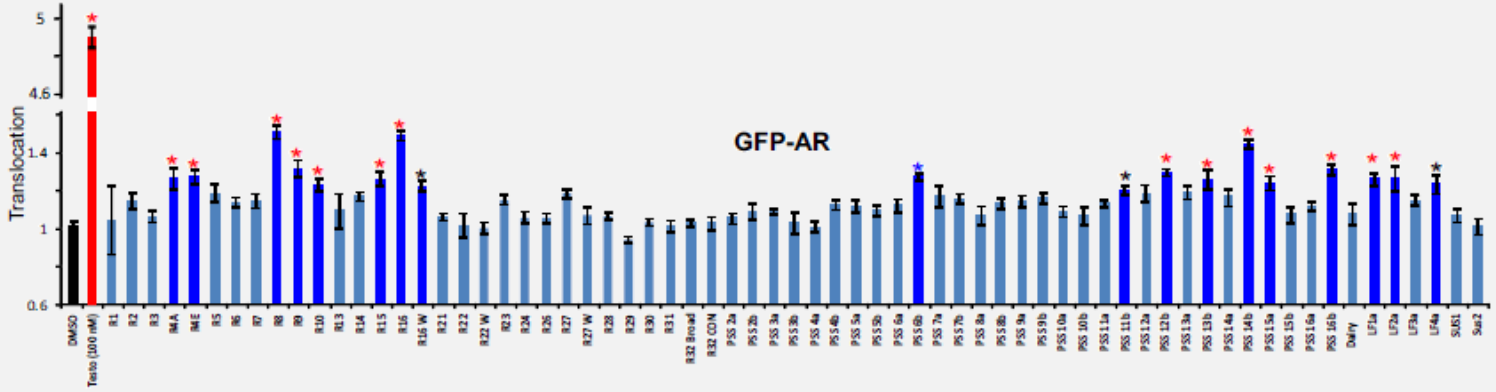


Automated screening of water samples for glucocorticoid and androgen activity by Opera



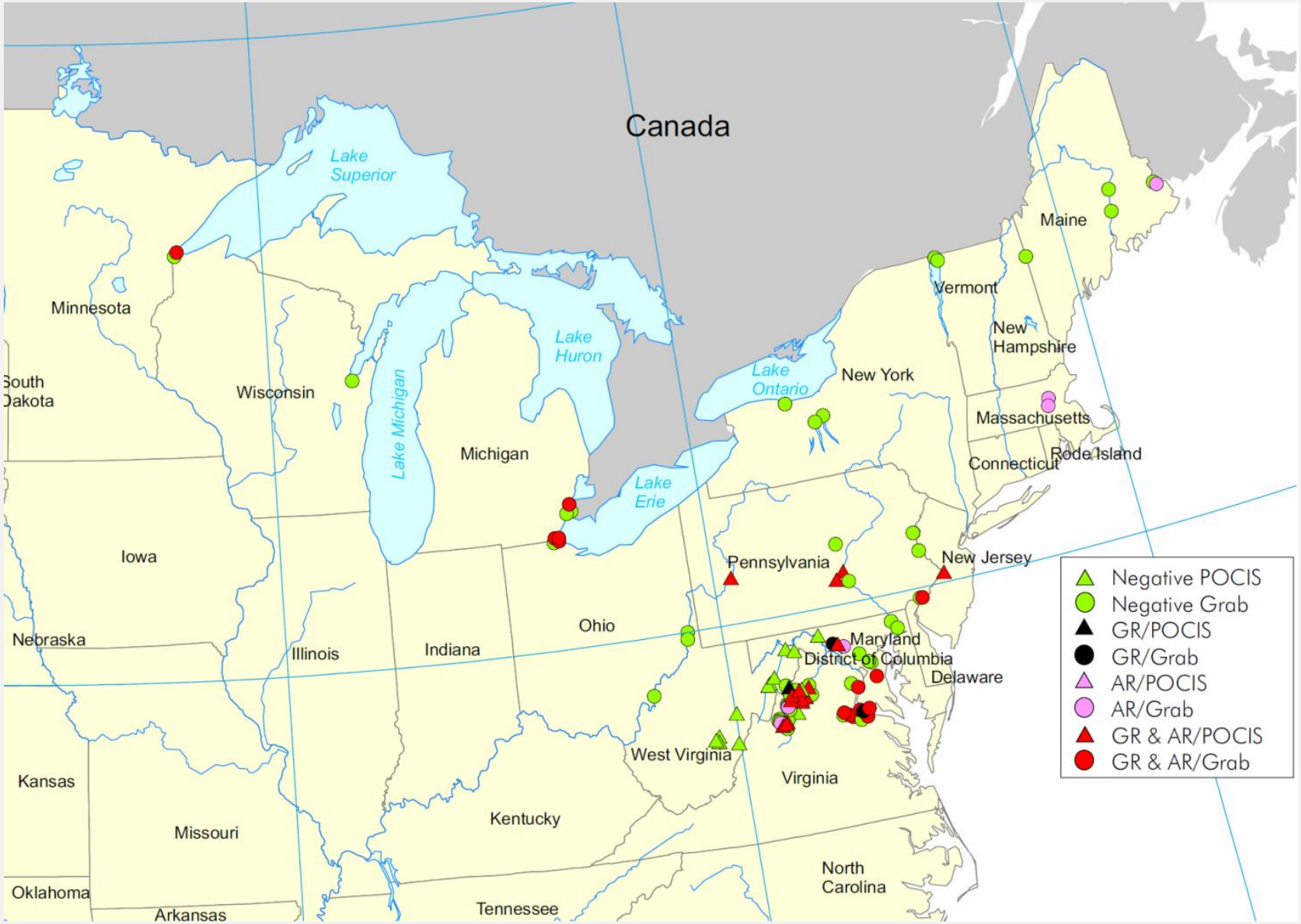






Samples: Luke Iwanowicz & Vicki Blazer, USGS-BRD

Geographic location of the collection sites and their contamination with glucocorticoid and androgenic activity



SCIENTIFIC REPORTS

OPEN

Prevalent Glucocorticoid and Androgen Activity in US Water Sources

Diana A. Stavreva [1], Anuja A. George [1*], Paul Klausmeyer[2], Lyuba Varticovski[1], Daniel Sack [1], Ty C. Voss [1], R. Louis Schiltz[1], Vicki S. Blazer [3], Luke R. Iwanowicz[3] & Gordon L. Hager [1]

Science Reports 2:1-8 (2012).

For over 100 sites examined in 14 states:

27% contained detectable glucocorticoid activity
35% contained detectable androgen activity

U.S. Patent Application No. 13/912,071
filed June 6, 2013
NIH (DHHS) Ref. No. E-269-2011/0-US-03
First Named Inventor: Hager
KITS FOR DETECTING AND MONITORING ENDOCRINE DISRUPTING CHEMICALS (EDCS)

Recent extension of screen to another 315 samples

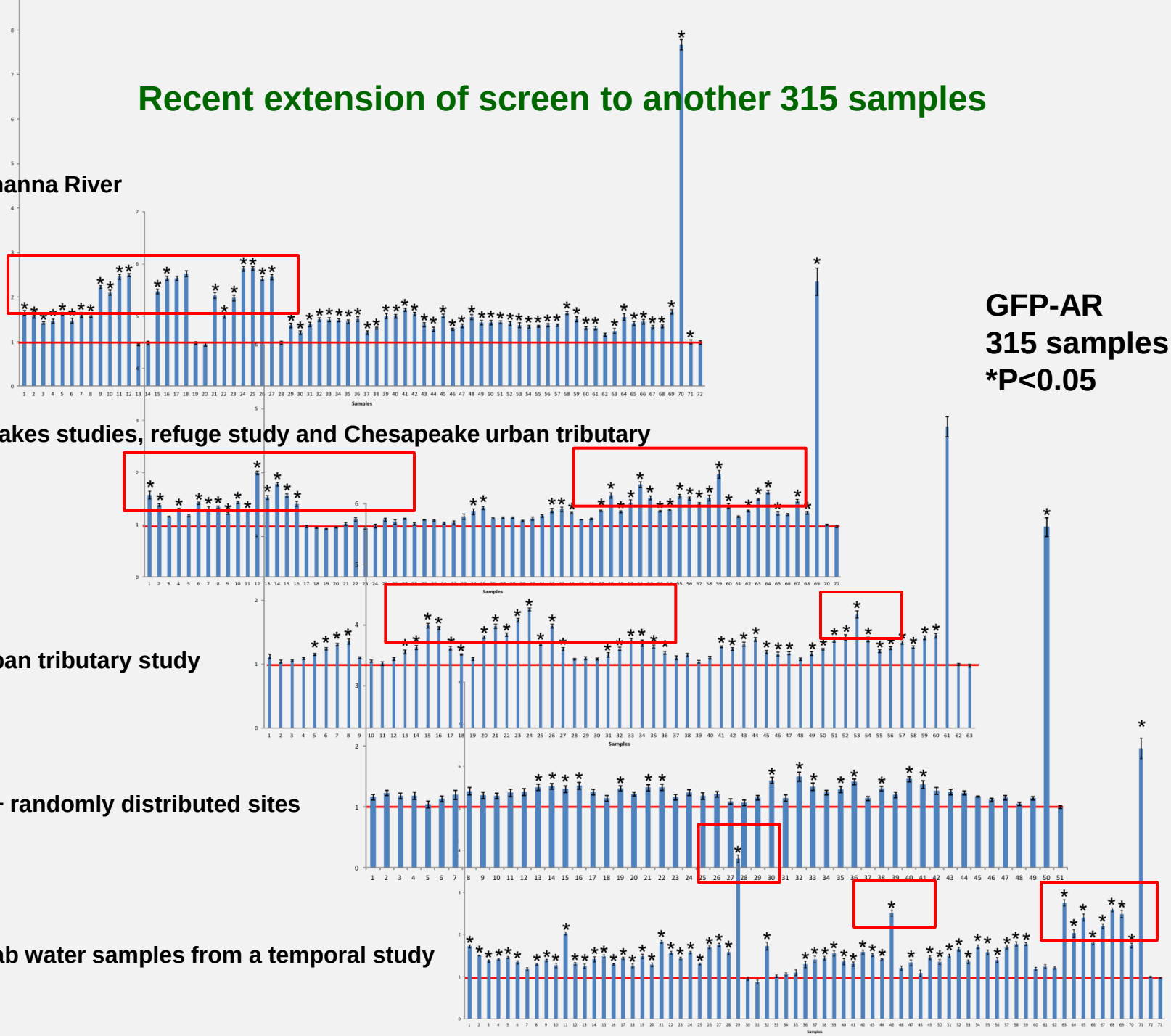
Susquehanna River

Great Lakes studies, refuge study and Chesapeake urban tributary

Urban tributary study

50+ randomly distributed sites

Grab water samples from a temporal study



GFP-AR
315 samples
***P<0.05**

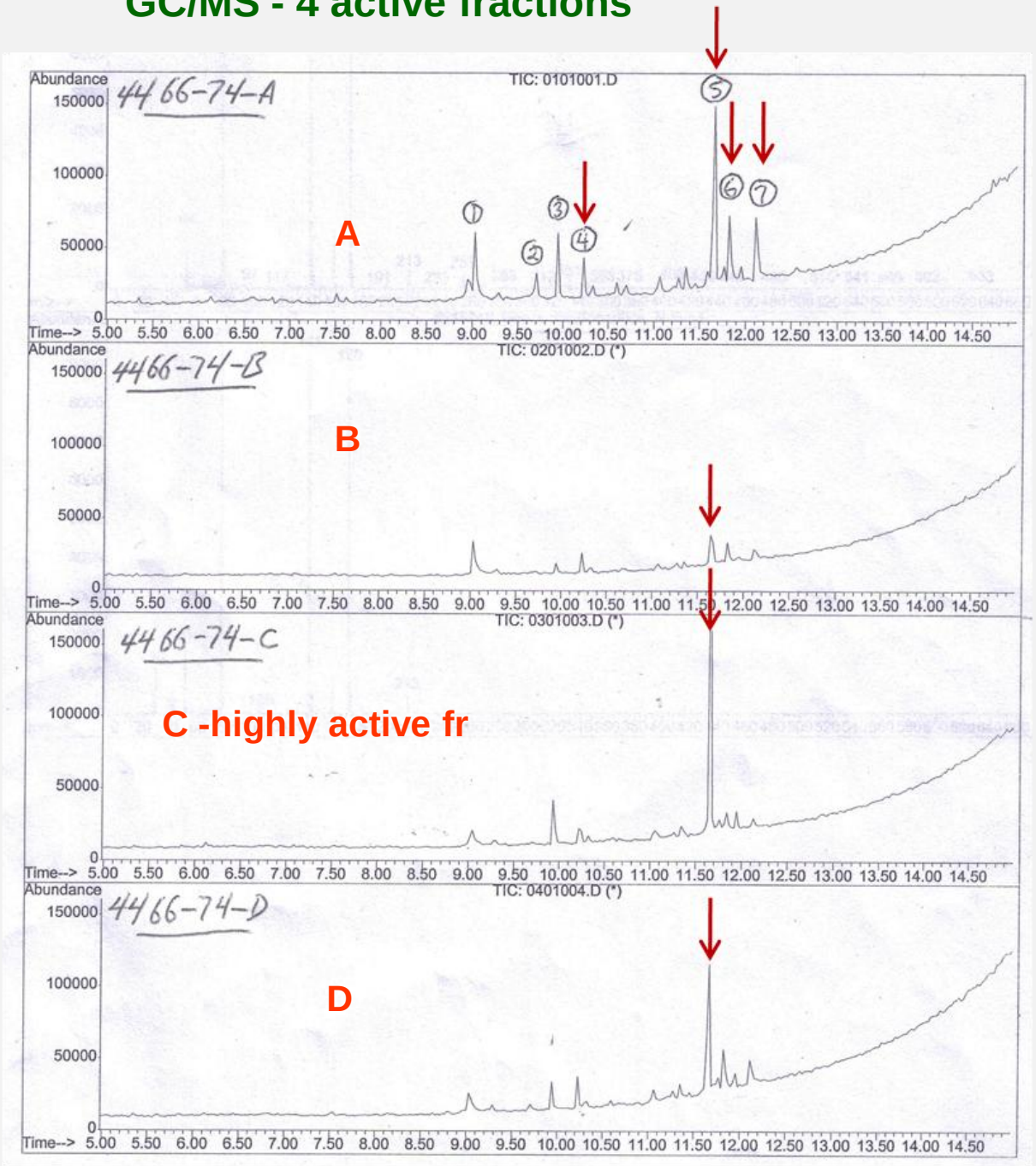
Major issue:

What is the actual molecule active at a given site?

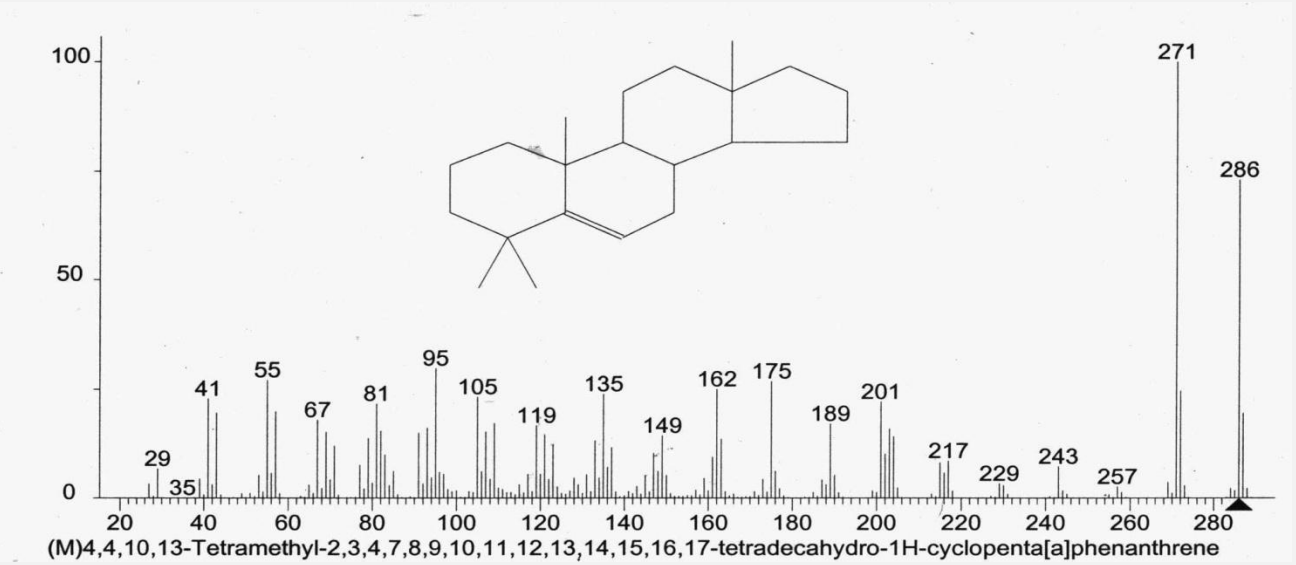
GC/MS - 4 active fractions

What is the active component?

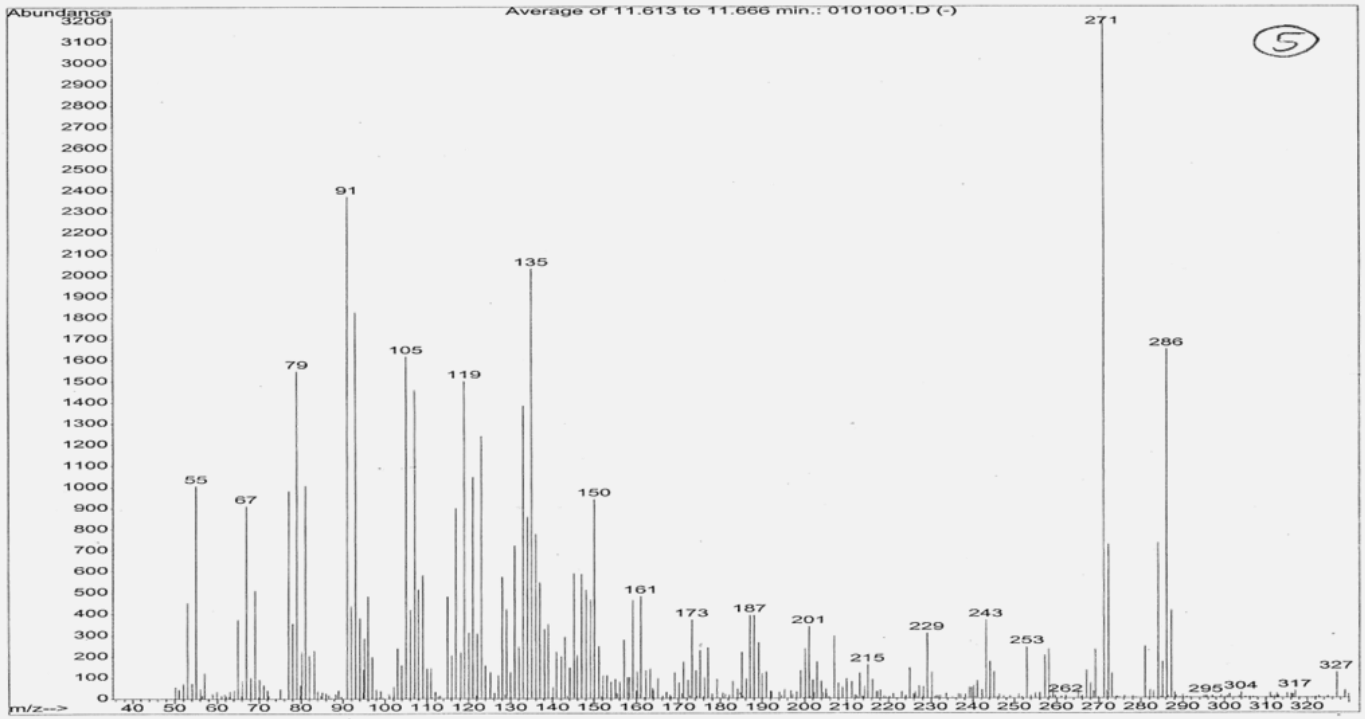
Peak #	MW
4	272
5	286, 343
6	270, 288
7	288



1. Possible match for peak 5 – GC/MS data

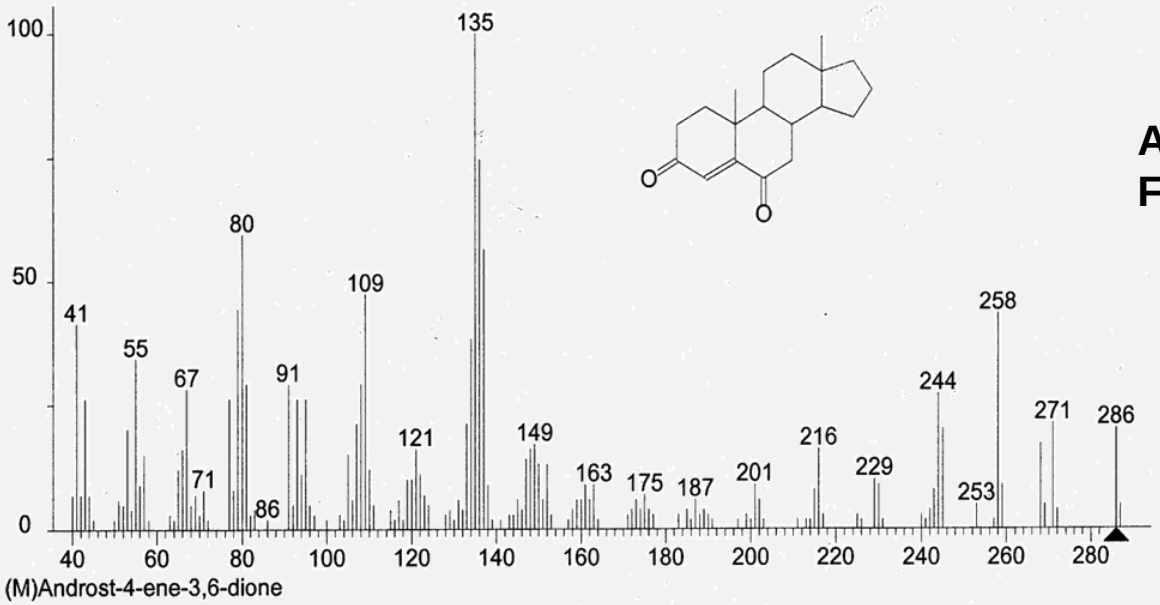


4,4-dimethyl-androst-5-en
From the NIST database



Fr 97, peak 5

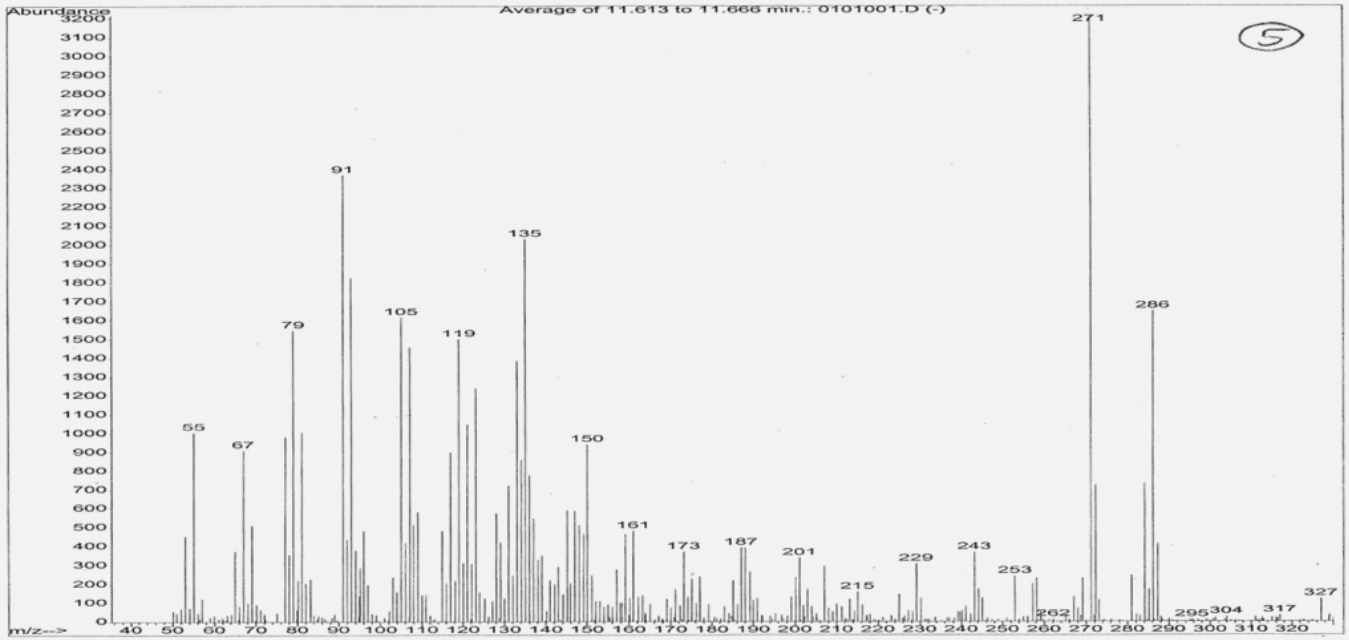
2. Possible match for peak 5 – GC/MS data



Androst-4-ene-3,6-dione,
From the NIST database

No clear identification

Water samples are a zoo of variants



Fr 97, peak 5

Another complexity:

Sciencexpress

Product-to-Parent Reversion of Trenbolone: Unrecognized Risks for Endocrine Disruption

Shen Qu,[1] Edward P. Kolodziej,[2]* Sarah A. Long,[3] James B. Gloer,[3] Eric V. Patterson,[4] Jonas Baltrusaitis,[5,6] Gerrad D. Jones,[2] Peter V. Benchetler,[2] Emily A. Cole,[2] Kaitlin C. Kimbrough,[2] Matthew D. Tarnoff,[1] David M. Cwiertny[1,7]*

Science 342:347-351 (2013)

Trenbolone acetate:
Synthetic anabolic steroid used as growth promoter in US cattle industry (20 million cattle per year)

Subjected 17-trenbolone to multiple light & dark cycles in the lab

Concentrations fell during simjulated daytime, but <u>rebounded during the dark cycles</u>

nature International weekly journal of science

NATURE | NEWS

Hormone disruptors rise from the dead

Broken-down pollutants reform in the dark, casting doubt on environmental risk assessments.

"…Hormone-disrupting chemicals may be far more prevalent in lakes and rivers than previously thought"

"…..Endocrine disruptors - pollutants that unbalance hormone systems - are known to harm fish, and there is growing evidence linking them to health problems in humans, including infertility and various cancers. But pinpointing specific culprits from the vast array of trace chemicals in the environment has proved difficult."

"…current environmental monitoring procedures still rely on checking "a list of chemicals, and they only know how to look for one thing at a time", he says."

Conclusions:

Potential contamination of U.S. waterways with EDCs that act on nuclear receptors is largely unstudied

Detectable levels of corticosteroid and androgen activities can be measured with surprising frequency

Are these levels of significance in relation to human health? do they persist in water supplied to the population?

Are there low level effects?

Screening assays for specific chemicals, i.e. a specific hormone, are of little value in addressing this issue

Rapid conversion of a complex molecules to many forms in the environment indicates the need for activity assays, not specific chemical assays